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POLLUTION ECOLOGY STUDIES OF THE POTEAU-POTTAWATOMIE WATERS

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Temperature had an effect on population dynamics, percent of females with eggs, number of eggs per female, and sexual reproduction. Egg production rates were estimated and observations on the duration of egg development were made.

This report also includes a field study of the relation between *Polyarthra vulgaris* and 19 selected chemical and physical parameters.

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CULTURING AND ECOLOGY STUDIES OF THE ROTIFER, *POLYARTHRA VULGARIS*

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FOREWORD

Our nation's fresh waters are vital for all animals and plants, yet our diverse uses of water -- for recreation, food, energy, transportation, and industry -- physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota, develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life,

--to assess the effects of ecosystems on pollutants,

--to predict effects of pollutants on large lakes through use of models, and

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man.

This report describes conditions that affect survival and reproduction of the freshwater rotifer, ***Polyarthra vulgaris***.

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ABSTRACT

The results contained in this report represent research conducted to identify variables which affect the survival and reproduction of the rotifer, *Polyarthra vulgaris*. The following variables were studied: handling stress, container size, frequency of changing the culture medium, light quantity and quality, photoperiod, oxygen and vitamin requirements, fungal parasites, food preference and concentration, antibiotic effects of bluegreen algae, and temperature.

Temperature had an effect on population dynamics, percent of females with eggs, number of eggs per female, and sexual reproduction. Egg production rates were estimated and observations on the duration of egg development were made.

This report also includes a field study of the relation between *Polyarthra vulgaris* and 19 selected chemical and physical parameters.

This report was submitted in fulfillment of Grant Number R800815 by Virginia Polytechnic Institute and State University, and the research was partically supported by the Environmental Protection Agency. It covers a period from February 1, 1973, to June, 1974, and the work was completed in June, 1974.

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SECTION 1

INTRODUCTION

Rotifers are an important component of aquatic ecosystems and their role varies from herbivores to carnivores to detritivores to combinations of these types. Rotifers are preyed upon by larval fishes, copepods, other rotifers, and a variety of other invertebrates including protozoans. As such, they are important in energy transfer.

The rotifer, *Polyarthra vulgaris,* has been classified as a perennial, eurythermal species which exhibits population maxima in the late spring or early summer (Carlin, 1943; Pejler, 1957). From the research of Edmondson (1965), Dieffenbach and Sachse (1911), Pejler (1957), and others, *P. vulgaris* has been identified as a herbivore feeding primarily on the alga *Cryptomonas.* In limited studies rotifers have been used to culture larval fish (Theilacker and McMaster, 1971; Harada, 1970; Maksinova, 1969). Siefert (1972) studied the first food of the yellow perch, white sucker, bluegill, emerald shiner, and the rainbow smelt. Based on electivity indices he concluded that *Polyarthra* were highly selected initial prey for the yellow perch and the bluegill. This information on feeding was significant for these primary reasons: (1) if the rotifer *Polyarthra* could be cultured, it would facilitate the laboratory culturing of bluegills or yellow perch, (2) the sensitivity of *Polyarthra* to toxicants in nature could be important in determining a successful year class of the larval fish that preyed upon it, and (3) the effect of starvation could be diminished when conducted acute and chronic bioassay studies on fish to determine application factors.

Polyarthra vulgaris, while perennial, was most dominant in late spring and it was never abundant most of the year for research purposes. To our knowledge, this rotifer has never been cultured in the laboratory. This inability to culture the animal stems from a limited knowledge of the animal and of the factors which affect its reproductive success.

The objectives of this study were to:

1. Determine what parameters affect survival and reproduction of the rotifer

2. Determine if healthy cultures of the rotifer could be maintained in the laboratory

3. Study a field population with emphasis on chemical and physical parameters.

This project was supported by a grant (R800815) from the Environmental Protection Agency. This is the final report for this grant.

The grant was awarded for one year and extended for five months without additional funds.

SECTION 2

CONCLUSIONS

With reference to objectives 1 and 2 and the results of this preliminary research, the following culture conditions were favorable for *Polyarthra vulgaris:*

1. Handling - the rotifers should not be handled any more than necessary.
2. Containers - glass containers containing a large volume of water, at least one liter.
3. Culture medium - water from a natural source that contains *Polyarthra vulgaris.* The water should be filtered through a 30 micron mesh net to remove larger algae and animals.
4. Replacing medium - partially twice a week and totally once a week.
5. Light - an incident illumination of 400 to 500 ft-c, a complete light spectrum, and a 16L:8D photoperiod.
6. Oxygen - moderate aeration to maintain a concentration near 8 ppm.
7. Vitamins - minimally the vitamins B_{12}, thiamine, Biotin, and pantothenic acid may be required by the rotifer. To cultures containing five liters of water, one-half gram of Vionate was added after each partial change of culture medium and one gram was added after each complete change.
8. Food type - Rotifers fed on a mixture of *Chilomonas paramecium, Cyathomonas truncatus, Bodo minimus, B. variabilis,* and *B. mutabilis* which was raised in a Purina trout chow medium and fortified with vitamins B_{12}, thiamine, biotin, and pantothenic acid.
9. Food quantity - 50 ml of this protozoan mixture was added to a 5-liter culture daily and the protozoan concentration was around 300,000 protozoans per ml.
10. Temperature - within a few degrees of 20 C.
11. Antibiotic and parasitic agents - Bacteria of the *Sphaerotilis-Leptothrix* complex, fungi, and dense populations of green and bluegreen algae were detrimental to the rotifer.
12. Population density - if the rotifer density decreased below 40 animals per liter the population usually did not recover.

Objective 2 was partially achieved and cultures were maintained for 70 to 100 days at room temperature (21 $\pm$ 2 C) and at 20 C. Because the populations cycled and because densities far exceeded natural levels, we had good culture success but more research is needed.

Studies on *Polyarthra vulgaris* population dynamics and reproduction at different temperatures have been evaluated and the following conclusions reached:

1. Reproduction does not occur at 10 and 30 C but it does at 20 C and room temperature (21 ± 2 C).
2. At or near 20 C population densities may exceed 20,000 per liter.
3. The percent of ovigerous females was lowest when population densities were highest.
4. Ovigerous females usually carried one egg but they could exceed three per female. Multiple eggs usually occurred after the population peak.
5. Sexual eggs appeared after a population peak.
6. Estimated egg production rate varied from 0.08 to 0.25 eggs per day.
7. Time for egg development may exceed 24 hr at room temperature and this may be due to a fluctuating temperature.

In fulfillment of objective 3, the following parameters significantly correlate with field populations of *Polyarthra vulgaris:*

1. Photoperiod - positive correlation
2. Temperature - positive correlation
3. Oxygen - negative correlation (illusory)
4. Ammonia - negative correlation
5. Nitrate - positive correlation (possibly illusory)
6. Orthophosphate - positive correlation (possibly illusory)
7. Sodium - negative correlation
8. Total filtrable solids - negative correlation

Significant variables identified by the stepwise regression analysis were temperature, photoperiod, orthophosphate, silicon, magnesium, and nitrates. The other parameters were variable in their effect and not significant.

SECTION 3

RECOMMENDATIONS

Much more research is needed to optimally culture *Polyarthra vulgaris* for future studies. These needs are:

1. *Polyarthra* should be cultured, if possible, in an artificial medium. This would reduce the antibiotic effects of algae and protozoans found in pond water and the negative effects of naturally occurring chemicals such as high ammonia and sodium concentration.

2. The vitamin requirements of zooplankton are poorly understood. The results of this study suggests that the B vitamins in solutions were a major factor controlling successful reproduction and growth.

3. Because the vitamin enriched water also enhanced the growth of fungi and bacteria, research needs to be conducted on the possible use of antibiotics to reduce infestations that reduce population success.

4. The food organisms (protozoans) used in this study were adequate for culturing. However, more research is needed to identify and culture more suitable food organisms. In recent work *Dinobryon sp.* may be the major natural food for *Polyarthra*. (Buikema, unpublished).

5. Because natural systems are thermally labile, research is needed to determine if *Polyarthra* reproduction is enhanced by fluctuating temperatures rather than by static temperatures.

6. Studies should be conducted on the effects of periodic harvesting of *Polyarthra* to determine optimum harvesting rates for minimizing population fluctuations.

SECTION 4

FACTORS AFFECTING SURVIVAL AND REPRODUCTION OF POLYARTHRA VULGARIS

Rotifers have been cultured in filtered natural waters (Edmondson, 1960, 1964a; Halbach, 1970a, 1970b; etc.), in defined media (Gilbert, 1963, 1970; Maly, 1969; King, 1967; Laderman and Gutlman, 1963; Meadow and Barrows, 1971; Lynch and Smith, 1931; Shull, 1911; Finesinger, 1926; Buikema, Cairns, and Sullivan, 1974), and in undefined media (Pennak, 1953; Dougherty, 1960, 1963; Maksinova, 1969; Halbach, 1970a) which generally were natural waters innoculated with milk, dried greens, etc. Algal media were not satisfactory (Adachi, 1964; Lansing, 1942, 1947). The rotifers which were cultured were both littoral and limnetic, and commonly cultured genera include *Asplanchna, Brachionus, Monostyla, Keratella,* and *Kellicottia* (Edmondson, 1960, 1964; Gilbert, 1963, 1968; Chu, 1934; Halbach, 1970a, 1970b, 1972; Maly, 1969; Laderman and Gutlman, 1963; Dewey Bunting, pers. comm.; etc.).

Numerous problems culturing *Keratella* and *Kellicottia* were reported by Edmondson (1960). These problems included vessel size, shape, and material.

Food was an extremely important variable and two factors were important: (1) the size of the food particle, and (2) the nutritive value of the food. Particle size is important for *Polyarthra* (Edmondson, 1965; Gossler, 1950) and populations of *Polyarthra* have been positively correlated with the cryptophyte *Cryptomonas* (Edmondson, 1965; Pejler, 1957; Dieffenbach and Sachse, 1911; Pourriot and Hillbricht-Ilkowska, 1969) but Carlin's data (Figures 50 and 101 in 1943) show considerable variation between populations of *Cryptomonas* and *Polyarthra*. This alga varies from 15 to 80 μ in length and 8 to 18 μ in width (Prescott, 1951) while the rotifer is 130 to 150 μ in length (Bartos, 1959). The feeding observations by Dieffenbach and Sachse (1911) were for *Polyarthra platyptera* and *P. euryptera* (Hutchinson, 1967) and *Cryptomonas ovata*. The studies by Pourriot and Hillbricht-Ilkowska (1969) were for *Polyarthra trigla* and *Cryptomonas curvata*. The correlation of Edmondson (1964b) was for *Polyarthra vulgaris* and smaller species of *Cryptomonas* (14 x 31 μ). Smaller sized organisms probably were not eaten by *Polyarthra vulgaris* (Edmondson, 1965). Food quality and quantity are known to influence population dynamics of rotifers (Pourriot, 1957; King, 1967; Halbach, 1972).

Supplemental vitamins added to the culture medium may be necessary for rotifer culture. Vitamin supplementation enhanced egg production of the copepod, *Tigriopus* (Shiraishi and Provasoli, 1959) and of *Daphnia* (Fritsch, 1953). Dougherty, Solberg, and Harris (1960) suggest that bacteria may provide essential nutrients for rotifers. But algae may also because they produce

vitamins B_{12}, biotin, and thiamine (Carlucci and Bowes, 1970a, 1970b).

Temperature is probably the most important factor affecting rotifer reproduction and development. Positive correlations between temperature and limnetic rotifers have been observed by many workers (Kolisko, 1938; Edmondson, 1960, 1964, 1965; King, 1967; Halbach, 1970a; Pourriot and Hillbricht-Ilkowska, 1969; Tauson, 1926; and others). The temperature optimum for *Polyarthra vulgaris* is probably between 10 and 20 C (Edmondson, 1965; Carlin, 1943) although Carlin suggested that there is a population peak in the fall when the temperatures are between 5 and 10 C. Duration of life span is also temperature dependent and rotifers generally live longer at lower temperatures (Kolisko, 1938; Edmondson, 1945; etc.). Egg development occurs faster at higher temperatures and for *Polyarthra vulgaris* it varies from 70 hr at 10 C (Edmondson, 1965) to 23 hr at 20 C. Acclimation also has an effect (Hillbricht-Ilkowska, 1969).

Light may also be an important factor. Long photoperiod or increased intensity may affect populations of *Kellicottia* (Edmondson, 1965), and rotifers such as *Polyarthra* behaviorally respond to light (iaud, 1943; Hutchinson, 1967; etc.). Light intensity may be an important factor affecting organisms as it has been suggested for *Daphnia pulex* (Buikema, 1972, 1973a, 1973b, 1975).

Hutchinson (1967) summarized the possible chemical variables that may affect rotifer populations. The pH may affect rotifer populations (Edmondson, 1944; Adachi, 1964; Harring and Meyers, 1928; Lansing, 1942; Myers, 1931; Ahlstrom, 1940), and Edmondson (1944) suggests that there may be more than one factor which produces the apparent limitation due to pH. Pejler (1957) concluded that pH was of no real importance in the distribution of rotifers from northern Sweden. Not very much is known about planktonic organisms (Hutchinson, 1967). Oxygen concentration may affect populations (Whitney, 1917, 1919; Tauson, 1925; Adachi, 1964) and egg hatching (Lite and Whitney, 1925) although some species are able to live in oxygen deficient water (Pejler, 1957; Beadle, 1963). Carbon dioxide, bicarbonate concentration, and calcium can affect reproduction (Tauson, 1925, 1926, 1927; Lansing, 1942) and distribution of some species (Hutchinson, 1967).

Birky and Gilbert (1971) summarize the literature of variables which control rotifer sexuality, and temperature, photoperiod, dissolved oxygen, pH, carbonate, food concentration, diet and population density can be controlled to limit mictic and amictic organisms.

MATERIALS AND METHODS

The initial series of experiments that were conducted with *Polyarthra vulgaris* were begun by estimating those variables (from the preceding literature) that could affect reproduction and survival and observing animals under these conditions in the laboratory in which these were varied.

The animals usually were collected from Pandapas Pond located 6 km northwest of Blacksburg, Montgomery County, Virginia, and on occasion from Carvins Cove Reservoir, Botetourt County, located 4 km north of Roanoke, Virginia.

Field collections were made with a 35 micron mesh net towed through the surface water. Concentrations of zooplankton were transported back to the laboratory in 18 liter polypropylene containers of pond or reservoir water. Cultures of animals were isolated from other zooplankton, placed in filtered pond or reservoir water, and slowly warmed to the experimental temperature.

Preliminary experiments were conducted at room temperature (21 ± 2 C) and at a long photoperiod (16L:8D). Later experiments were conducted in Scherer-Gilette CEL 4-4 growth chamber.

To our knowledge, this rotifer has never been cultured. Our preliminary experiments were based on simple observations of the rotifer under various conditions, and three criteria were used to indicate favorable conditions: (1) survival, (2) appearance of eggs, and (3) hatching of eggs. The following results and discussion summarize this preliminary research.

RESULTS AND DISCUSSION

Handling

This rotifer was quite sensitive to handling and death usually occurred within 8 to 12 hr after transfer. If the animal survived the first 12 hr it may survive for as long as 8 days. Handling with larger bore pipettes (1 mm) reduced death rate to a certain extent. Handling of the rotifers also increased the release of the egg (or eggs) which were carried by the female.

Culture Containers

Rotifers were placed in 0.5 mm depression slides, 5.0 ml shallow spot plates, 10.0 ml dish, 15.0 ml test tubes, 125.0 ml Erlenmeyer flasks, and 5.0 liter containers. Survival and egg production increased as the container size increased; this was most notable in the 5.0 liter container. All containers were glass except the 5.0 ml spot plate which was a polycarbonate plastic. The small glass containers were pyrex and the largest one was soft glass. A relationship between large containers and culture success has been demonstrated for the copepod *Diaptomus clavipes* (Robertson, Gehrs, Hardin and Hunt, 1974) and for rotifers (Edmondson, 1960). For *Polyarthra* the minimum size for a container appeared to be 10 ml.

Culture Water

Two sources of water were used for culture experiments (Table 1). This water came from sources that had *Polyarthra vulgaris* populations. Water was collected in 18-liter polypropylene containers and stored at room temperature for no more than 2 weeks. The water was filtered through a 35 micron mesh net and/or glass fiber filters prior to use. Pandapas Pond water was usually used.

The filtered water was used for setting up all the culture experiments. Two initial experiments were conducted to determine if the culture medium had to be replaced to insure culturing success. Survival and egg production

TABLE 1. CHEMICAL ANALYSIS OF CULTURE WATERS
(Except for pH, all values are in mg/l)

Parameter	Pandapas Pond Seasonal Range of Means	Carvins Cove*
Total Hardness ($CaCO_3$)	9.00 - 22.1	42 - 56
Total Alkalinity ($CaCO_3$)	6.20 - 31.4	48.0
pH	6.40 - 8.30	8.0
CO_2	0.00 - 17.30	1.0
Iron	0.04 - 2.15	0.02
Manganese	-- - --	0.01
Nitrate	0.00 - 0.57	0.0
Sulfate	0.40 - 4.80	16.0
Silicon	5.00 - 11.70	3.4
Magnesium	1.08 - 2.11	1.9
Calcium	0.96 - 4.86	20.04
Orthophosphate	0.00 - 0.09	--
Total Phosphate	0.002- 1.99	0.01
Potassium	0.99 - 1.86	1.18
Sodium	2.95 - 6.22	2.0
Ammonia	0.04 - 0.70	2.0
Total Filtrable Solids	17.20 -143.20	90.0

*December, 1972, from Roanoke City Water Authority.

increased if the water was replaced periodically. In our subsequent culture experiments, one-third of the culture water was changed every two days. The water was passed through a 30 micron net and the animals were returned to the aquarium. Once a week the entire water was replaced in each culture.

Light

Three lighting variables, photoperiod, light intensity, and wave length range, were tested for their effect on rotifer survival and reproduction. All experiments were conducted using GE cool white fluorescent bulbs (20 and 40 W). Three photoperiods were set up, 8L:16D, 12L:12D, and 16L:8D. The results appeared better under the longer photoperiod, but there were not statistically significant differences among the results.

The effect of light intensity on population numbers was also examined. The light intensities studied were 1500, 500, and 100 incident ft-c measured with a GE photometer. No detectable differences in population numbers were noted among the light intensities. However, when observations were made of the rotifers behavioral response in a light intensity gradient, maximum aggregation occurred at approximately 400 ft-c.

Wavelength effects also were examined using Rohm and Haas plexiglass filters and unfiltered light at 400 ft-c. Specifically the filters were #2400 (red), #2092 (green), and #2264 (blue). Respectively, the filter transmissions were 5.7%, 21.4%, and 2.9%. There were some differences among the light conditions (Table 2) and among the wavelengths; red was more favorable. In comparing the data between partial and full spectrum, population numbers increased more rapidly under the full spectrum.

Buikema (1973a) found that reproduction of *Daphnia pulex* was inhibited by red wavelengths and stimulated by blue wavelength. These results were opposite those obtained for *Polyarthra*. In comparing the effects of wavelength and light intensity on reproduction of *Daphnia pulex* the results were variable depending on the intensity examined and 14 ft-c was the best. These differences in numbers between light quality for *Polyarthra* may reflect light effects in survival as they may for *Daphnia pulex* (Buikema, 1973a).

Oxygen Requirements

The rotifers were very sensitive to oxygen deficiency and aeration was mandatory. Survival of the animals was less than 12 hr under low oxygen conditions. These results are consistant with field observations of the rotifers sensitivity to low oxygen (Abel, 1972; Pejler, 1957; this study).

Vitamin Requirements

Egg production was not observed in the laboratory until vitamin mixtures were added to the protozoan food medium which already contained No. 3 Purina Trout Chow (Table 3). The vitamins B_{12}, pantothenic acid, thiamine, and biotin promoted egg production (Table 4) and survival (Table 5) whether added as a mixture (#1) or as Vionate, a commercial pet food supplement.

Dieffenbach and Sachse (1911), Pejler (1957), and Edmondson (1965) have noted positive correlations between *Polyarthra vulgaris* populations and the alga *Cryptomonas*. We observed (see below) that *Polyarthra vulgaris* would not feed on *Cryptomonas ovata* - even though it meets the criteria of size and

TABLE 2. EFFECT OF RED, GREEN AND BLUE WAVELENGTH AND FULL SPECTRUM LIGHT ON *POLYARTHRA VULGARIS* POPULATIONS

Day	Number of Rotifers/Liter			
	Red	Green	Blue	Full Spectrum
0	200	200	200	200
1	120	80	320	160
3	40	160	80	120
6	80	160	0	160
8	0	80	120	120
10	80	120	100	40
13	80	200	200	560
15	160	200	160	680
17	40	40	160	920
19	40	40	<40	1000
21	<40	40	40	1920
23	120	<40	<40	3680
25	200	160	120	5080
29	760	<40	<40	3600
31	2480	<40	40	8080
33	3320	0	0	4280
35	3080	0	0	600

TABLE 3. VITAMIN CONTENT OF VARIOUS FOODS AND WATERS

Vitamin	Vionate* (dry per gram)	Vionate in pond water (conc./liter of culture water)	Purina #3 Trout food (dry per pound)†	Mixture #1 (conc./liter of culture water)
A	220.75 units	44.15 units	-	-
D_3	22.07 units	4.41 units	-	-
B_1 (Thiamine)	0.0397 mg	0.0079 µgram	33 ppm	0.20 mg
B_2 (Riboflavin)	0.0795 mg	0.0159 µgram	-	-
B_6 (pyridoxine)	0.01 mg	0.002 µgram	-	-
B_{12}	0.000155 mg	0.00031 µgram	18 gram	0.001 mg
Pantothenate	0.110 mg	0.022 µgram	160 ppm	0.20 mg
Niacin	0.276 mg	0.0552 µgram	-	-
Folic Acid	0.0022 mg	0.00044 µgram	-	-
Choline Chloride	5.7395 mg	1.1480 µgram	-	-
C	2.503 mg	0.500 µgram	-	-
E	0.120 units	0.024 units	-	-
Biotin	-	-	1.1 ppm	0.20 mg

*Vionate is made by Squibb and Sons, Inc. The diluent is soy grits, gelatin, sucrose, dried skim milk and corn germ meal.

†These are added amounts and they do not include natural concentrations in the peruvian menhaden fish meal.

TABLE 4. EFFECT OF VITAMIN MIXTURES ON POPULATIONS OF *POLYARTHRA VULGARIS* AFTER ONE WEEK
(Rotifers were fed vitamin enriched cultures of *Chilomonas paramecium, Cyathomonas truncata, Bodo minimus, B. variabilis* and *B. mutabilis)*

Vitamins	Initial population (number/liter)	Population after one week (number/liter)
None	200	<40
3 cc of Mixture #1	200	<300
3 cc of Mixture #1 plus mud	200	<40
2 gm of Vionate	200	300-600
3 cc of Mixture #1 and 2 gm of Vionate	200	<2000*

*eggs present

presumed food. Cryptomonads require B_{12} and thiamine for optimum growth (Hutchinson, 1967; L. Whitford, pers. comm.) and presumably so do the rotifers. It is possible that these two organisms occur together because of common requirements and not solely because *Polyarthra* is feeding solely on *Cryptomonas*.

The presence of trace elements may also have an effect because many are cofactors for enzyme activity. The concentrations present in Vionate and a mixture (#1) added to the water are in Table 6. There were no apparent effects when the mixture #1 trace elements were added to the culture. Data for Purina Trout Chow were not available.

Fungi and Bacteria

Unfortunately the high vitamin content of the culture water stimulated the growth of a chytridiaceous fungus and a sheath forming bacterium of the *Sphaerotilus-Leptothrix* complex. It was not uncommon to find adult rotifers and eggs enmeshed in fibers. Once enmeshed, rotifer death was certain. Contact with the fungi or bacterium was enhanced by the fact that the female rotifers released the developing eggs 12 to 24 hr prior to hatching. These eggs fell to the bottom of the culture container where the fungus or bacteria were growing on the debris or where there were spores.

TABLE 5. EFFECTS OF VARIOUS FOODS PLUS VITAMINS AND TRACE METALS ON SURVIVAL AND REPRODUCTION OF *POLYARTHRA VULGARIS*

Food		Vitamins and trace metals	Average number surviving per day: Day 0	1	2	3
A.	*Cryptomonas ovata*	no	10	0	-	-
	Cryptomonas ovata	yes	10	6	-	0
B.	*Ochromonas*	no	10	0	-	-
	Ochromonas	yes	10	6	-	0
C.	*Bodo sp.**	no	10	2	-	0
		yes	10	7	-	3
D.	*Chilomonas*	no	10	-	8	-
	paramecium and	no	10	2	-	0
	*Cyathomonas truncata**	yes	10	6	-	10
		yes	10	-	14	-
E.	3 species of *Bodo,*	no	10	2†	-	1†
	Pleuromonas and	no	10	-	6	-
	*Oikomonas**	yes	10	8	-	6
		yes	-	-	9	-
F.	no food	yes	10	4	-	0

*protozoans were raised on trout chow
†eggs present

One experiment was conducted with the wide spectrum antibiotic kanamycin sulfate. It was used at a concentration of 0.01 mg/liter which is 1/100 the level for treating *Philodina acuticornis* eggs (Meadow and Barrows, 1971). Even at this low concentration the antibiotic was toxic to the rotifer.

Fungal and bacterial growth was reduced if filtered Vionate solution was used rather than the straight compound in the culture water, but they were not eliminated.

Rotifer eggs are parasitized by fungi (Paterson, 1958; Seymour and Johnson, 1973) and one chytrid, *Olpidium gregarium,* has been identified from rotifer eggs (Paterson, 1958). Paterson also found that the rotifers *Notholca* and *Polyarthra* were parasitized by saprolegnianceous fungi. Fungi, but not bacteria, have been suggested for the decline in natural populations of *Polyarthra* (Beach, 1960; Pejler, 1961).

TABLE 6. ELEMENTS IN FOOD MEDIA AVAILABLE TO *POLYARTHRA VULGARIS*
(Data for Purina trout chow are not available)

Element	Vionate (per gm dry) weight	Mixture #1 Medium (µgm/l)
Ca	89.9 - 107.9	0.0
P	47.94	0.0
Na	5.0 - 15.0	0.0
I	0.022	0.0
Fe	0.552	0.0
Co	0.0055	0.0022
Cu	0.0552	0.0040
Mg	0.5298	0.0
Mn	0.0759	0.0041
Zn	0.0	0.0200
Mo	0.0	0.0019

Food

Because of the correlations between *Polyarthra* and the alga, *Cryptomonas*, initial experiments were conducted with this alga. Survival was poor indicating that the animals were not feeding on it. Six different culture media were used to raise *Cryptomonas ovata* (Table 7) at a recommended light intensity of 200 ft-c (Daniel Jones, pers. comm.). Basically the media were Bristol's and Chu's with different concentrations of nitrate and peptone. In all instances survival of the rotifers was still poor. Observations under the microscope indicated the the rotifer would grasp *Cryptomonas ovata* but then the rotifer would reject it. It was never observed eating *C. ovata*, even when there was no other choice. Also in subsequent studies *Polyarthra* was never observed to feed on *C. erosa* (W. Yongue, pers. comm.).

The size of the food particle was important for rotifers (Gossler, 1950; Edmondson, 1965), and other potential food sources were tested. Observations

TABLE 7. CULTURE MEDIA USED FOR *CRYPTOMONAS OVATA*

Medium	Additional Nitrogen mg/l	Peptone mg/l	pH
Chu's #10	750	0	7.8
Chu's #10	750	1	7.0
Chu's #10	250	1	7.0
Bristols	0	0	7.2
Bristols	250	1	7.1
Bristols	750	1	7.0

were made on the rotifer's selectivity for food under the microscope and on rotifer survival and appearance of eggs when the rotifer was placed in a 10 ml microcosm with various foods. Seventeen possible foods were made available to *Polyarthra vulgaris* (Table 8). Of these 17 foods the rotifer was observed eating 7 of them. Most other foods were ignored except *Cryptomonas ovata* which was grasped but then rejected by the rotifer. The incidence of feeding on *Euglena viridis* and *Chlamydomonas reinhardii* was much lower than it was for the non-chlorophyll containing forms.

The survival of *Polyarthra* increased markedly when they were raised on mixtures of *Chilomonas paramecium* and *Cyathomonas truncata* or three species of *Bodo* (Table 9). Interestingly, *Chilomonas* and *Cyathomonas* are also cryptophytes as are *Cryptomonas* and *Rhodomonas*, possible foods suggested for *Polyarthra* (Edmondson, 1965; etc.). Additionally egg production and limited egg hatching occurred with these food mixtures even though no vitamins were added to the food. Survival was also best if vitamins were present with the food (Table 5).

Food concentration had some effect on survival (Table 10). Generally survival was better at the lower food concentration. At these lower food concentrations the number of protozoan per ml of food stock varied between 80,000 to 350,000. The study of Erman (1962) suggest that high food concentrations are necessary. *Brachionus calyciflorus* eats up to 180% of its wet weight in wet food per day (Erman, 1962). Unfed *Polyarthra* usually died within 8 to 12 hr although some lived for up to 24 hr (Table 10). Food concentration does affect reproduction of *Euchlanis* (King, 1967), *Brachionus* (Halbach, 1972), and other rotifers (Edmondson, 1965; etc.).

TABLE 8. POTENTIAL FOOD ORGANISMS TESTED ON *POLYARTHRA VULGARIS*

Taxa	Size (in microns)	Selected by animal
1. *Euglena viridis*	14-20 x 40-65	yes
2. *Cryptomonas ovata*	508 x 20-80	no
3. *Paramecium aurelia*	50-60 x 120-180	no
4. *Paramecium bursaria*	50-60 x 100-150	no
5. *Phacus sp.*	c. 25 x 50	no
6. *Trachelomonas sp.*	c. 15 x 30	no
7. *Chlamydomonas reinhardii* (#89,+)	3-5 x 10-15	yes
8. *Chlamydomonas moewusii*	10-15 x 15-20	no
9. *Cyathomonas truncata*	10-15 x 15-25	yes
10. *Chilomonas paramecium*	10-15 x 15-25	yes
11. *Bodo variabilis*	5-15	yes
12. *Bodo minimus*	c. 5	yes
13. *Bodo mutabilis*	5-15	yes
14. *Pleuromonas jaculans*	5 x 10	no
15. *Oikomonas termo*	5 - 10+	no
16. *Ochromonas sp.*	5 - 30	no
17. *Chlorella sp.*	5 - 10	no

These data on food selectivity, survival, and reproduction of *Polyarthra* do not agree with the observations of Dieffenbach and Sachse (1911), Gossler (1950), Pejler (1957), or Edmondson (1965). For one, *Polyarthra vulgaris* did not feed on *Cryptomonas* and in its presence the rotifer did not survive for

TABLE 9. EFFECTS OF VARIOUS FOODS ON SURVIVAL AND EGG PRODUCTION OF *POTAMOTHRA VULGARIS*

Food	Day	Average Number Surviving Per Day												
		0	1	2	3	4	5	6	7	8	10	11	15	17
Cryptomonas		10	-	1.5	1.0	-	-	-	-	-	-	-	-	-
Cryptomonas		10	-	1.5	-	-	-	-	-	-	-	-	-	-
Cryptomonas		10	-	2.0	-	-	-	-	-	-	-	-	-	-
Cryptomonas		10	-	4.0	3.0	1.0	-	-	-	-	-	-	-	-
Cryptomonas		10	-	0.3	-	-	-	-	-	-	-	-	-	-
Cryptomonas		10	-	-	-	-	-	-	-	-	-	-	-	-
Chilomonas and		10	3.0	1.0	-	-	-	-	-	-	-	-	-	-
Cyathomonas		12	-	-	-	11	-	-	-	4.0	2.0*	3.0*	2.0	1.0
Cryptomonas,		10	-	3.0	-	-	-	-	-	-	-	-	-	-
Chilomonas, and		10	-	2.0	-	-	-	-	-	-	-	-	-	-
Cyathomonas		10	1.0	-	-	-	-	-	-	-	-	-	-	-
Chlamydomonas moewusii		10	1.5	-	-	-	-	-	-	-	-	-	-	-
Euglena viridis		10	8.0	4.0	-	-	-	-	-	-	-	-	-	-
Trachelomonas		10	3.0	-	-	-	-	-	-	-	-	-	-	-
3 species of *Bodo*,		10	2.0*	-	-	-	-	-	-	-	-	-	-	-
Pleuromonas and		10	3.0	-	-	6.0*	5.0*	4.0*	2.0	2.0	1.0	-	-	-
Oikomonas		10	9.0	-	-	-	-	-	-	-	-	-	-	-
Ocromonas		10	3.0	-	-	-	-	-	-	-	-	-	-	-
Ochramonas and *Bodo sp.*		10	2.0	1.0	-	-	-	-	-	-	-	-	-	-
Bodo sp.		10	2.0	2.0	-	-	-	-	-	-	-	-	-	-

*Eggs present; development occurred in some

TABLE 10. EFFECT OF VARIOUS CONCENTRATIONS OF PROTOZOANS (NO VITAMINS) ON SURVIVAL AND REPRODUCTION OF *POLYARTHRA VULGARIS*

Food	Relative Concentrations	Average Number Surviving per Day						
		Day 0	1	2	3	4	5	6
A. None	-	10	-	0	-	-	-	-
		10	10	0	-	-	-	-
		10						
B. *Euglena*	2X		7.5	3	2*	-	-	-
	5X	10	2	1	0	-	-	-
	10X	10	7	2.5	0	-	-	-
C. *Chilomonas* and *Cyathomonas* (Run 1)	2X	10	6	5.5	5.5	6*	-	1
	4X	10	8	-	-	-	-	1
	5X	10	9	11	3.5	2	-	0
	10X	10	8	8	2	1	-	0
C. *Chilomonas* and *Cyathomonas* (Run 2)	1X	10	-	3	2	-	-	-
	2X	10	-	4	0	-	-	-
	3X	10	-	0	-	-	-	-
	5X	10	-	3	2	-	-	-
	10X	10	-	3	0	-	-	-
	20X	10	-	3	0	-	-	-
D. 3 species of *Bodo, Pleuromonas* and *Oikomonas*	1X	10	0	-	-	-	-	-
	2X	10	0	-	-	-	-	-
	3X	10	2	2	20	-	-	-
	5X	10	1	0	-	-	-	-
	10X	10	1	0	-	-	-	-
	20X	10	2	0	-	-	-	-

*eggs present

long periods of time much less produce eggs. Earlier we proposed an alternative hypothesis for the field correlations between *Cryptomonas* and *Polyarthra vulgaris* based on their common requirements for vitamins B_{12} and thiamine. Secondly, *Polyarthra* will feed on small sized protozoans such as *Bodo* in contrast to the observations of Edmondson (1965) although he acknowledges that *Polyarthra* was "not utterly dependent on *Cryptomonas*."

In our study the rotifers fed most frequently on the colorless flagellates and reproduction occurred when this food source was available. Edmondson (1965) did not find a significant correlation between *Polyarthra vulgaris* and colorless flagellates. There are two possible reasons for this: (1) the colorless flagellates he examined were less than 10 μ long and 2 of our food organisms, *Chilomonas* and *Cyathomonas*, were larger; and (2) his analysis of the colorless flagellates was not species specific but rather a composite of various protozoans. In both instances we would not expect a significant correlation to appear in his analyses.

Antibiosis

Possible inhibitory effects of algae on *Polyarthra* populations were observed. Concurrent with declines in populations of *Polyarthra* there were increases in numbers of specific algal genera. In five of the seven instances recorded the dominant genera were bluegreen algae. These included *Anabena, Spirulina, Phormidium,* and *Oscillatoria*. In two instances the dominant genera were green algae, especially *Ankistrodesmus, Eudorina, Pandorina* and *Pediastrum*. Direct or indirect antibiotic effects of *Chlorella* have been suggested for populations of *Kellicottia longispina* (Edmondson, 1965), *Brachionus plicatus* (Hirayama, Watanabe, and Kusano, 1973) and *B. calyciflorus* (Halbach, 1972; Halbach and Halbach-Keup, 1974) and similar relationships may exist with other rotifers and algae. In comparing Carlin's data for *Oscillatoria* and *Polyarthra vulgaris* populations (Figures 145 and 146 in Hutchinson, 1967) there may be an inhibitory effect because the rotifer population was low when the *Oscillatoria* population was high. Similar inhibitory effects may occur for the bluegreens, *Aphanizomenon* and *Lynbya* (Figures 54, 56, and 101 in Carlin, 1943). Bluegreen algae may even affect the vertical distribution of *Polyarthra* (Figures 53, 55, 57, and 100 in Carlin, 1943).

Temperature

Preliminary experiments were conducted on temperature and survival. *Polyarthra vulgaris* seems to be resistant to wide temperature fluctuations. For example, animals can be taken from the field at 3 C and slowly warmed to 20 C within 5 hr with no apparent effects. Some rotifers lived as long as 48 hr.

Initial population experiments were conducted at 10 and 20 C and at room temperature (21 + 2 C). These population experiments were conducted prior to refinement of culture techniques, but they provided some insight into temperature effects. Two cultures were begun at 10 C and three each at 20 C and room tempeature. The rotifers were obtained from 25 to 26 C pond water. These data are summarized in Figure 1. All populations died within 40 days.

Population success was best at 20 and 21 C and was least at 10 C. These observations were consistent with the field observations of Carlin (1943), Pejler (1957), and Edmondson (1965).

Egg Development

Incomplete observations were made on egg development at room temperature

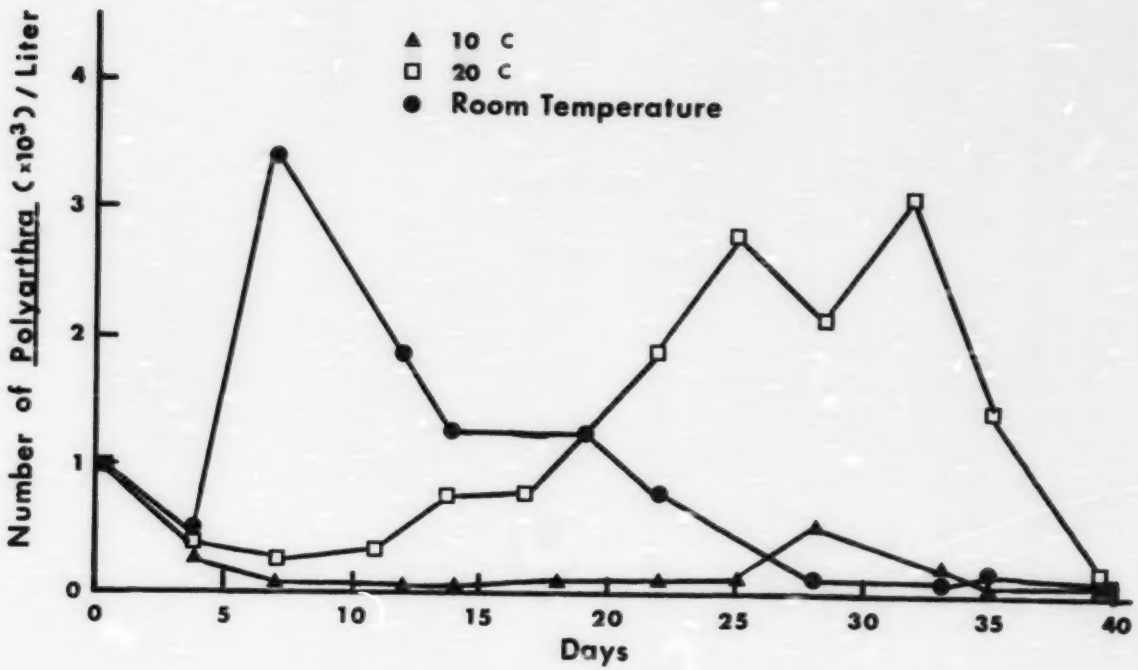

Figure 1. Effect of Temperature on Populations of *Polyarthra vulgaris*.

(21 + 2 C) incidental to our other work. Pourriot and Hillbricht-Ilkowska (1969) demonstrated that egg development of *Polyarthra vulgaris* can occur within 24 hr at 20 C and that it can take up to 70 hr (Edmondson, 1965). Such rapid development is commonly accepted for parthenogenetic zooplankton, especially the rotifers. In our observations we did not notice such rapid development. At room temperature (21 ± 2 C) development took much longer than 24 hr since observations were begun with ovigerous females. In one instance a female already bearing an egg was observed in a 10 ml microcosm and the egg hatched 8 days later. The young rotifer was morphologically similar to the female with distinct paddles and it appeared to be healthy.

There are many possible reasons for this extended development time of the egg. Maternal nutrition may be an important factor. Edmondson (1965) suggests that egg laying of rotifers is influenced by light conditions and perhaps the same may be true for egg development. Another hypothesis, on constant versus fluctuating temperatures on egg development, is proposed in Section 5. Edmondson's (1965) calculations of duration of egg development were in part based on the assumption that the animals were found at a fixed temperature, and his duration studies were conducted at the "same temperature in which the animal has been living." Because the surface water temperatures where the animals were found fluctuated daily and because the rotifers respond to changes in light intensity (=vertical migration), we would expect that the rotifers were subjected to a range of temperatures each day even if this range were small.

The egg was attached to the female during part of the development time. It has been our experience that the egg was released by the female about 12 hr prior to hatching. The egg settled to bottom of the container where hatching occurred. In a lake it probably would remain in suspension. This egg release may have an effect on the calculation of egg ratio and reduce estimates of reproductive rates (Edmondson, 1960). The handling of *Polyarthra* also causes a release of egg, and the egg ratio would be further reduced.

SECTION 5

THE EFFECTS OF TEMPERATURE ON REPRODUCTION OF *POLYARTHRA VULGARIS*

The previous research on *Polyarthra vulgaris* had been concerned with the identification of variables and their effects on survival, egg production, and egg hatching. The purpose of this experiment was to optimize all the known variables except the temperature and study the effect of temperature on population dynamics. Temperature was chosen because it was a significant variable identified in this research and that of others (Edmondson, 1965; Edmunds, 1974; and Pejler, 1957).

MATERIALS AND METHODS

Populations of *Polyarthra vulgaris* were obtained from Pandapas Pond during October, 1973, when the water temperature was between 15 to 20 C. The animals were concentrated with a 35 micron mesh net and then added to the aquaria at an initial concentration of approximately 1000 rotifers per liter.

Filtered Pandapas Pond water was used for the cultures and five liters were placed in six-liter soft glass aquaria (Carolina Biological Supply Company, Burlington, North Carolina). One and one-half to two liters of the culture water were replaced twice a week and the water was completely replaced once a week. During the changes the rotifers were collected in a Wisconsin net bucket with a 30 micron mesh bolting cloth and returned to the aquarium. The debris at the bottom of each aquarium was removed each week and discarded.

Vitamins were added to the cultures in two ways. First, after each complete water change 1.0 gm of Vionate (Squibb and Sons, Inc.) was added to the water. An additional 0.5 gm of Vionate was added after each partial change of water. Secondly, vitamins were supplied through the protozoan cultures. Protozoan food organisms were cultured on dilute #3 Purina Trout Food which also contained vitamin mixture #1 (Table 3).

The protozoan cultures were mixed and contained *Chilomonas paramecium, Cyathomonas truncata, Bodo mutabilis, B. variabilis,* and *B. minimus*. Fifty ml of this protozoan mixture was fed to each culture each day. The concentration of protozoans was about 300,000 protozoans per ml.

Concurrently three cultures were placed in each of four temperature conditions: 10, 20, 30 C, and room temperature (21 + 2 C). The first three temperatures were regulated in Scherer-Gilette Cel 4-4 growth chambers and

temperature was regulated within 0.75 C.

The aquaria were aerated moderately to maintain the dissolved oxygen concentration in excess of 8 ppm. The photoperiod was a 16L:8D cycle with the incident light intensity between 100 and 500 ft-c (from one end of the aquarium to the other). The light sources were 20 and 40 watt GE cool white fluorescent bulbs.

Five 5-ml subsamples were removed every 2 to 3 days from each aquarium with a pipette and counted. The population counts are probably underestimated because 22% of *Polyarthra* can escape a glass tube such as an eye dropper (Szlauer, 1965). If available, 10 to 20 freeswimming females were examined for eggs, and notes were made on the percent of females carrying eggs and the number of eggs per ovigerous female. The occurrence of sexual eggs was noted. After counting the eggs, 10% formalin was added to each subsample and the total number of rotifers was counted. Formalin caused egg release so it was necessary to count eggs on living rotifers.

RESULTS

The population data for the 4 temperatures are depicted in Figures 2 and 3. Population success was poor at 10 and 30 C, and there was more variation among the cultures at 30 C than at 10 C (Figure 2). Reproduction was depressed at 10 C and no rotifers were present after 13 days. The populations at 30 C exhibited reproduction within 5 days with a decline in population numbers until day 10. Population maxima exceeded 10,500 rotifers per liter. No rotifers were present after day 10. There was a significant bloom of bluegreen algae in the 30 C cultures and the dominant genera were *Anabena, Spirulina, Phormidium,* and *Oscillatoria*. An antibiotic effect has been suggested earlier (Section 4). No eggs were observed in the 10 and 30 C cultures.

The best results were obtained at 20 C and room temperature (Figure 3). There was considerable variability among the cultures at both of these temperatures, and only the mean values for three cultures are presented. While mean population numbers were as high as 7,500 rotifers per liter, individual cultures at 20 C were above 11,000 rotifers per liter and at room temperature rotifer concentration exceeded 13,000 per liter. In other experiments at 20 C populations greater than 20,000 per liter were observed.

The populations at 20 C exhibited 3 major peaks which were 22 and 28 days apart, while only 2 major peaks, 37 days apart, were observed at room temperature (Figure 3). The data in Figure 3 are for 69 days, but 1 rotifer culture at room temperature lasted longer than 100 days. Five of the 6 cultures crashed after the water was changed on the 70th day, and it was believed that a toxic substance was present in the pond water. Usually the population numbers did not rebound if the rotifer density fell below 40 animals per liter.

The data in Figures 4 and 5 illustrate representative patterns between population numbers, percent of ovigerous females, number of eggs per ovigerous female, and appearance of sexual eggs. The percent of ovigerous females was

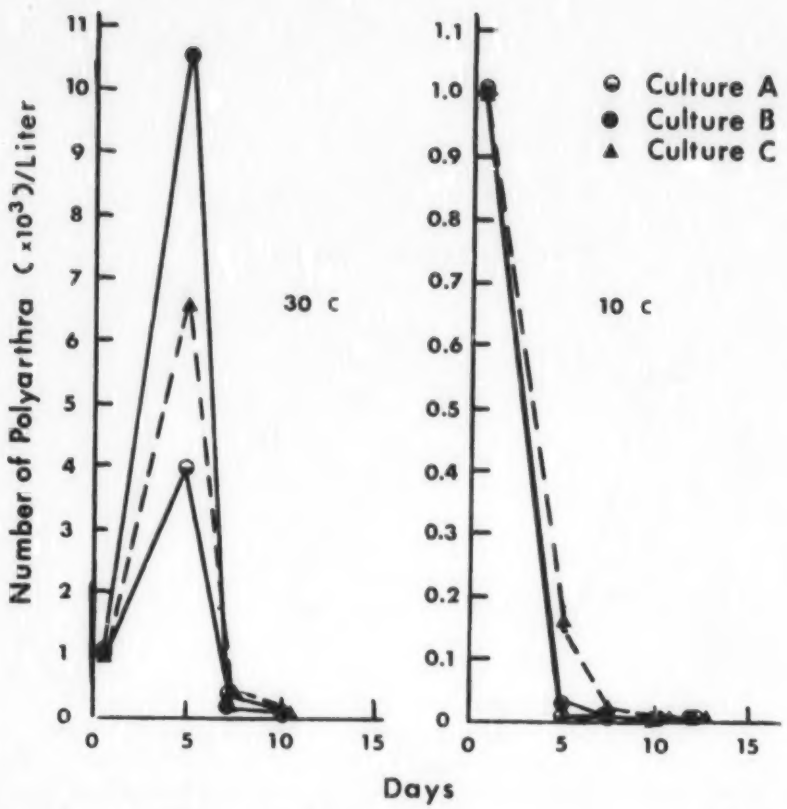

Figure 2. Effect of 10 and 30 C Temperatures on Populations of *Polyarthra vulgaris*.

always greatest when population numbers were low with a general decrease after population peaked. There were large fluctuations in the percent of ovigerous females, and it varied from 0 to 70% when population numbers were low (Figure 4). Similar fluctuations occurred in other populations. Generally the fluctuations in percent of ovigerous females was regular (up and down) in 20 C cultures but not in room temperature cultures. In these cultures dips may persist from two to five days (e.g., Figure 5, days 22 to 27).

Ovigerous females usually carried one egg at a time. Two eggs per female were not uncommon and rarely did a female carry three eggs. In one instance a female with 5 eggs was observed in a 20 C culture. The average number of eggs per ovigerous female usually increased after a population peak (Figure 4). This phenomenon was observed five times. Only once was the average number of eggs per ovigerous females greather than one prior to a population peak (Figure 5).

When there were multiple eggs they usually were smaller than the single egg, but not as small as would be expected for male producing eggs. Males

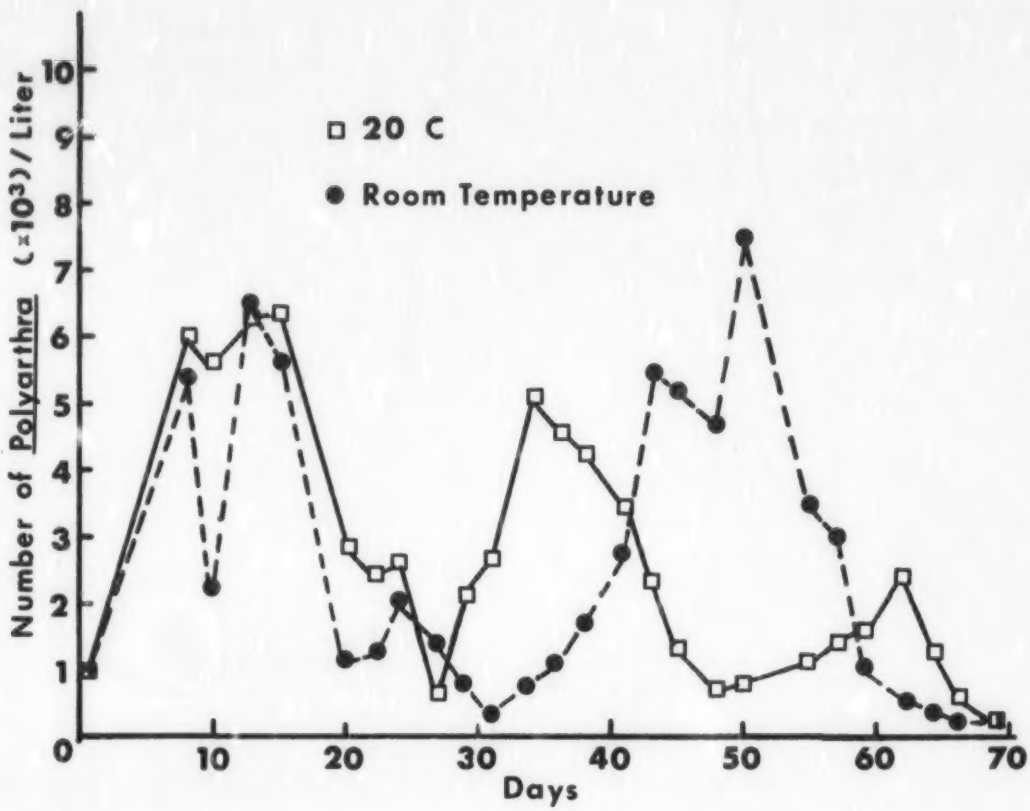

Figure 3. Effect of 20 C and Room Temperature (21 ± 2 C) on Populations of *Polyarthra vulgaris*.

were not observed, but their presence was indicated by the appearance of sexual eggs. These eggs were large and darkly pigmented. Sexual eggs were only observed twice (Figures 4 and 5), and in both cases it was after a population peak.

DISCUSSION

In comparing the data at 10, 20, and 30 C the results are comparable to data of others. Reproduction of rotifers is greatest at higher temperatures (Edmondson, 1960, 1965; etc.). The 30 C may be above the critical thermal maxima even though *Polyarthra vulgaris* was reproducing in 26 C water (Section 6, this study). Suppression of reproduction at 10 C corresponds favorably with Edmondson's (1965) observations on reproductive rate.

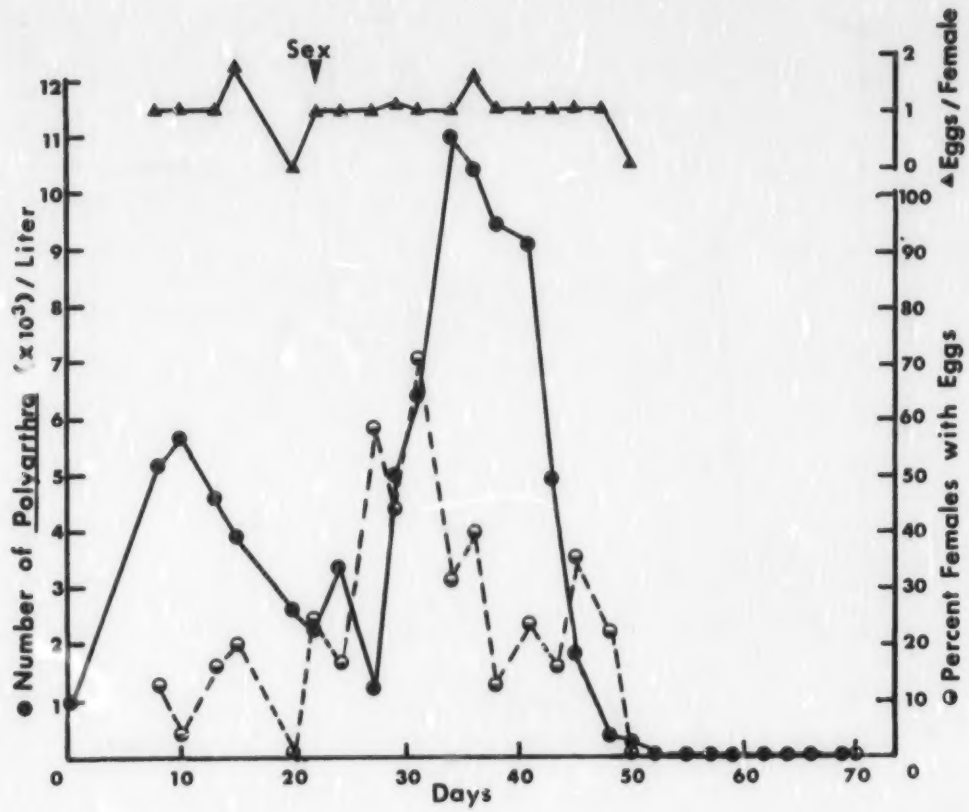

Figure 4. Relationship among Population Numbers, Percent Ovigerous Females, Number of Eggs per Ovigerous Female and Sexual Reproduction for One Culture of *Polyarthra vulgaris* at 20 C.

The variation in data obtained at 20 C and room temperature was interesting. Small sample size definitely influences our data such that differences in time between population peaks may not be significant. However, an analysis of the data suggest real temperature effects. Even though the cultures were conducted concurrently at similar temperatures with similar treatment, there was one difference that could have affected the results. The temperature fluctuation of the 20 C cultures was about 0.75 C or a range from 19.25 to 20.75 C. The room temperature cultures varied from 19 to 23 C over a 24 hr period for a 4 C range. The effect of a 4 C oscillation in temperature may have had an effect on rotifer reproduction and development. Halbach (1973) noted that variable temperature had the following impact on populations of *Brachionus calyciflorus:* immaturation time decreased; life duration increased; the intrinsic rate of population increase was larger, the environmental capacity was higher, and population fluctuations were more severe.

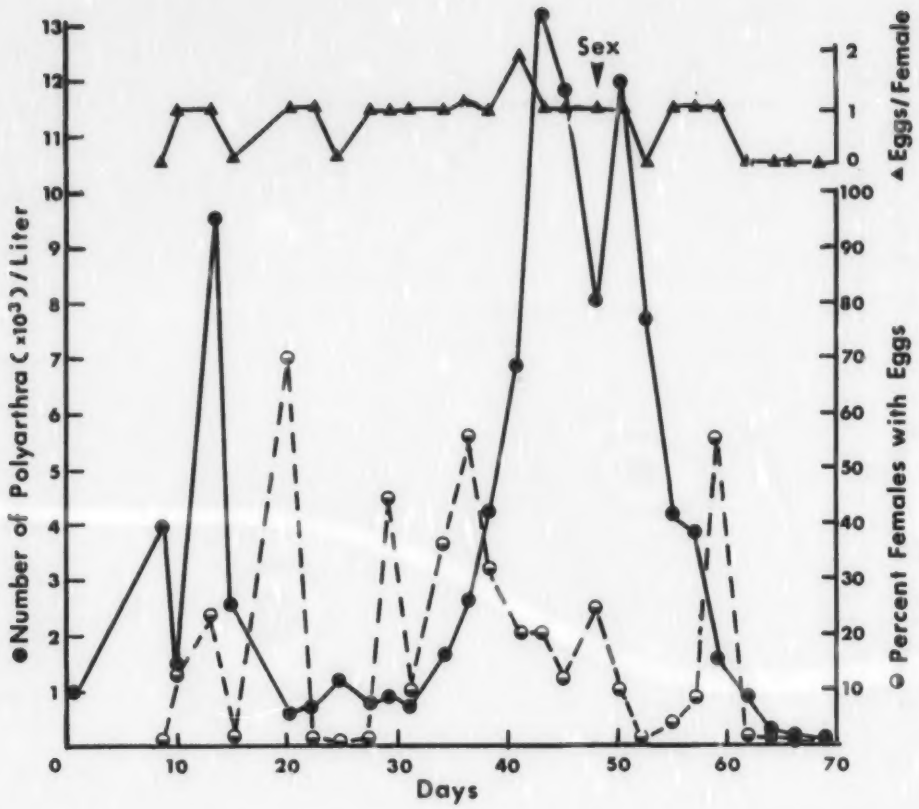

Figure 5. Relationship among Population Numbers, Percent Ovigerous Females, Number of Eggs per Ovigerous Females and Sexual Reproduction for One Culture of *Polyarthra vulgaris* at Room Temperature.

Egg production of plankton rotifers has been studied by Edmondson (1960). Values for *Keratella cochlearis* may vary from 0.077 to 0.267 eggs per day. The higher values were obtained at higher temperatures. While daily observations on specific females were not possible at this stage of the research, an analysis of the percent of ovigerous females provides a crude index (Figures 4 and 5). At 20 C the time between peaks was from 4 to 7 days. Because most females carried 1 egg, the rate of egg production at 20 C probably varies from 0.14 to 0.25 eggs per day. At room temperature the peaks were 7 to 12 days apart and the rate ranged from 0.08 to 0.14 eggs per day. These figures were similar to the values obtained by Edmondson (1960), but they also identify another difference between the 20 C and the room temperature cultures.

Our observation that the time for egg development at room temperature exceeded 24 hr (Section 4, this study; Edmondson, 1965; Pourriot and Hillbricht-Ilkowska, 1969) also suggests that the oscillating temperature may retard development. Acclimation of female *Polyarthra vulgaris* definitely has an effect

on egg development (Pourriot and Hillbricht-Ilkowska, 1969) which almost doubled when unacclimated animals were taken from 8 C and placed at 20 C. The data suggest that the temperature interaction was via the female prior to or during egg laying. Perhaps an oscillating temperature prior to egg laying may also prolong development.

Fluctuating temperatures are known to reduce the oxygen consumption of *Oncopeltus* eggs to levels below those of eggs held at an equivalent constant mean temperature (Richards and Suanraksa, 1962). Lower metabolic rate would suggest a retardation of development. Unfortunately, *Oncopeltus* eggs reared at a fluctuating temperature hatched 10% faster, but this same effect may not be true for parthenogenetically reproducing females subjected to a fluctuating environment. Hutchinson (1967) suggests that "maternal influences of a biochemical kind may have a greater effect on development in the rotifers..." and he cites data from Lansing (1942) on calcium and rotifer aging.

The decrease in the percent of ovigerous females when population numbers were high illustrates the classic textbook pattern of a feedback mechanism controlling population size and reproduction.

While sexual eggs were observed twice, males were not. Carlin's (Figure 101 in 1943) data for *Polyarthra vulgaris* show that in natural populations males appear only in the autumn and not during or after populations peaks as they do in other species of *Polyarthra*. From our data we suggest that males may appear after very large populations blooms. Our laboratory population densities were over 40 times greater (Figure 5) than the natural populations studied by Carlin (1943) and up to 13 times greater than the maximum recorded densities in our pond studies (1115/liter). At lower natural population levels, environmental parameters such as decreasing photoperiod or temperature may be more appropriate triggers for male formation.

SECTION 6

NATURAL POPULATION DYNAMICS OF *POLYARTHRA VULGARIS*

The laboratory culture research was based on the data from many literature sources. These data included information on a few possible chemical and physical parameters and food organisms. Because of the limited information available, we conducted a field investigation to (1) substantiate the observations by earlier works, and (2) examine additional chemical and physical parameters of the water that may effect populations of *Polyarthra vulgaris*.

MATERIALS AND METHODS

Description of Study Area

Populations of *P. vulgaris* were studied in Pandapas Pond, a shallow man-made impoundment located in the Jefferson National Forest, Montgomery County, 6 km northwest of Blacksburg, Virginia, at an altitude of 664.8 m above sea level (Figure 6). The maximum depth of the pond was 3 m adjacent to the 100 m earthen dam. The surface area is approximately 32,376 m^2 and the volume is estimated at 37,000 m^3 (U. S. Soil Conservation Service, pers. comm.). The pond is surrounded by steep (15 to 45 degree) slopes which are heavily forested with second growth pine hardwood forest overlaying a shallow, acidic shaley loam. Several small spring-fed streams feed Pandapas Pond, but the majority of the water is from runoff. The pond was generally devoid of aquatic insects and aquatic vegetation. The dominant fish was the bluegill, *Lepomis macrochirus*, and the smallmouth bass, *Micropterus dolomieui*, was present. In late spring the pond was the breeding place for large numbers of the newt *Notophthalmus viridescens*.

Sampling and Analytical Techniques

Studies began four months before the grant began and continued for eight months during the grant period. Samples were obtained monthly at two stations in the pond (Figure 6). Station One was located at the shallow end and Station Two was near the earthen dam.

An ITT JABSCO electric pump was used to collect all samples through a hose with a bell-ended "T" apparatus (Welch, 1948). Samples were taken for plankton, water chemistry, bacteria, and chlorophyll a.

Twenty liters of water were concentrated through 35 and 75 micron mesh nets for plankton. The 75 micron mesh net was used throughout the study, and

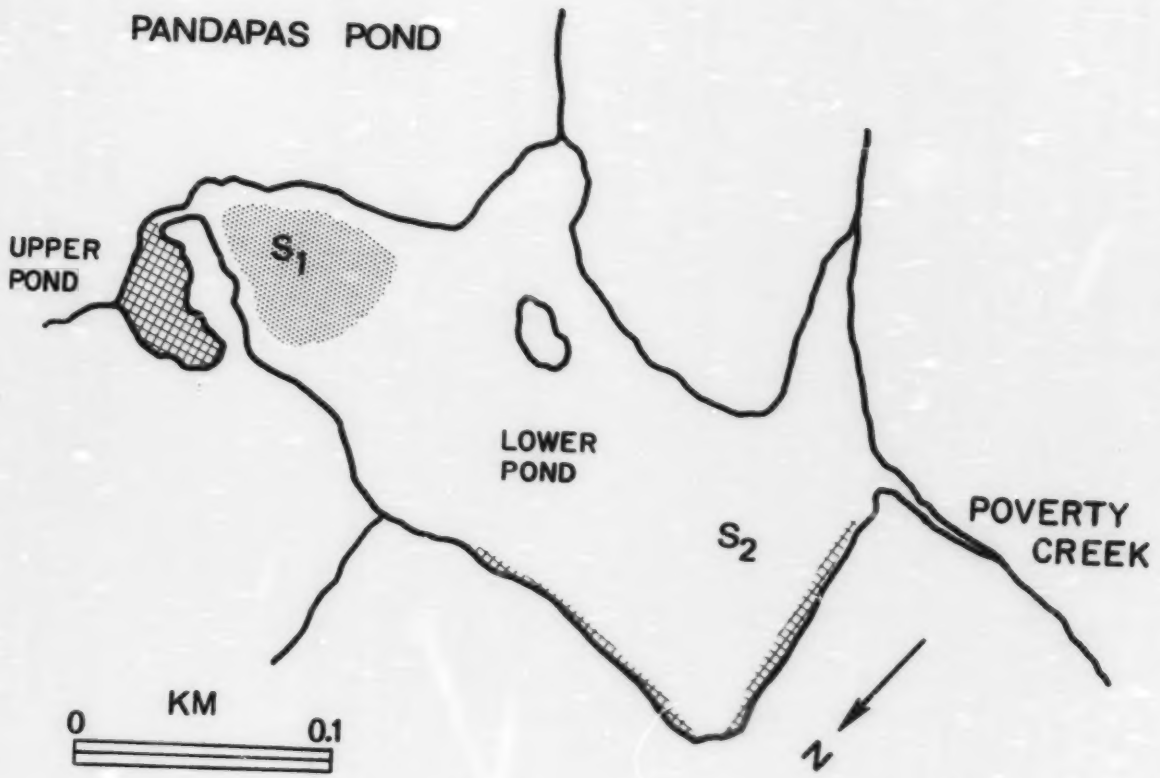

Figure 6. Map of Pandapas Pond, Montgomery County, Virginia Showing the Two Collection Stations (S_1 and S_2). Also Indicated are a Bed of *Spirogyra* (Stippled) and Beds of *Nitella* (Hatched).

the 35 micron net was used for the last 7 months of the study. [illegible] samples were preserved in 10% formalin and counted at a later date w[illegible] a Sedgewick-Rafter counting cell.

Nineteen chemical and physical parameters were determined for each sample. Temperature was measured with a YSI model 54 oxygen-temperature meter. Light penetration was obtained with a G.M. Submarine Photometer (GM Manufacturing and Instrument Corporation, Bronx, New York). Dissolved oxygen was measured with azide modification of the Winkler method. Oxygen samples were fixed in the field and titrated immediately upon return to the laboratory. All other water samples were iced and analyzed in the laboratory.

The pH was measured with a Corning model 109 meter. Conductivity was measured by a YSI conductivity meter. Nitrate and ammonia nitrogen, ortho- and total phosphate, sulfate, alkalinity, and total filtrable solids were measured by the methods outlined in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1971). Calcium, magnesium, iron, sodium, and potassium were measured on an Unicam Model SP90 Atomic Absorption Spectrophotometer. Total hardness was calculated from the calcium, magnesium, and iron values (APHA, 1971), and silica was measured by a Hach Chemical Kit (Hach Chemical Company, Ames, Iowa).

Samples for bacterial analysis were collected in sterile flasks and iced immediately. In the laboratory these samples were diluted and incubated as outlined in the Standard Plate Count method (APHA, 1971). The plates were counted after 24 hr incubation at 35 C using a Quebec darkfield colony counter. The number of colonies per ml of pond water was calculated.

Chlorophyll a samples were buffered with magnesium carbonate and iced. In the laboratory they were extracted and analyzed by the methodology of APHA (1971) and Strickland and Parson (1968).

The above data were subjected to correlation and multiple regression analyses using the Statistical Analyses System (SAS) of Barr and Goodnight (1972) on an IBM 370 Computer. These analysis were with no lag, which is appropriate for parthenogenetically reproducing animals (Angino, Armitage and Saxena, 1973).

RESULTS

Pond Chemistry

A detailed discussion of the yearly trends in pond chemistry can be found in a MS thesis by Edmunds (1974), and the discussion is summarized in Tables 11, 12, 13, and 14. Pandapas Pond was essentially a low hardness, low alkalinity system with the pH varying from 6.1 to 8.7 over the year. Yearly temperatures ranged from 0.1 to 26.0 C, and there was evidence of summer stratification at Station Two. Oxygen varied from 0 to 15.4 mg/l with obvious depletion during the summer at Station Two. Nitrate levels varied from 0 to 1.59 mg/l and ammonia values varied from 0.04 to 1.27 mg/l. Orthophosphate varied from 0.08 to 0.24 mg/l and the total phosphate was usually below 0.5

TABLE 11. SIGNIFICANT CORRELATIONS BETWEEN ENVIRONMENTAL PARAMETERS AND *POLYARTHRA VULGARIS* AT STATION 1

Environmental Parameter	Net Mesh (microns) 35	75	range mean	low	high
Photoperiod	+0.664*	+0.365*	12.14	9.67	14.73 hr
Temperature	+0.510*	+0.461*	12.73	0.10	26.00 C
Nitrate	+0.463**	NS†	0.21	0.00	0.65 mg/l
Orthophosphate	+0.422**	NS†	0.03	0.00	0.14 mg/l
Oxygen	-0.407††	-0.366††	9.33	4.90	12.80 mg/l

Significant levels:

*.005
**.01
†not significant
††.05

mg/l except in June when a high value of 12.6 mg/l was found at 3.0 m in Station Two. Sulfates ranged between 1.4 to 4.81 mg/l. Calcium levels (0.96 to 3.82 mg/l) were similar to magnesium levels (1.10 to 3.07 mg/l) while iron values varied between 0.04 to 2.15 mg/l. Total hardness ranged from 7.49 to 22.08 mg/l. Sodium values varied (2.95 to 6.22 mg/l) while potassium values were usually between 1.0 to 2.0 mg/l. Total alkalinity varied from 5.1 to 31.4 mg/l. The concentration of total filterable solids fluctuated irregularly from 17.2 to 143.2 mg/l. Silicon was always present in concentrations above 4.7 mg/l, and the values were as high as 19.0 mg/l. Carbon dioxide varied from 0 to 100 mg/l. Conductivity was measured 3 times and it ranged from 28 to 39 micromohs/cm. Surface penetration of light ranged from 19.8 to 91% and because of high turbidity the percent penetration dropped markedly by 1.0 m.

Bacteria

The results of the bacterial analysis were too variable and unreproducible to be included in this study.

Chlorophyll a

Mean chlorophyll a concentration varied widely over time with a general

TABLE 12. NONSIGNIFICANT CORRELATIONS BETWEEN ENVIRONMENTAL PARAMETERS AND POPULATIONS OF *POLYARTHRA VULGARIS* at STATION 1

Environmental Parameter	Net Mesh		Concentration		
	35	75	mean	high	low
Carbon dioxide	-0.259	-0.147	3.24	0.00	11.00 mg/l
Total Filtrable Solids	-0.271	-0.171	52.40	4.00	192.00 mg/l
Sulfate	-0.241	-0.234	2.93	0.00	6.14 mg/l
Sodium	-0.233	-0.233	5.04	4.20	8.35 mg/l
Total Alkalinity	-0.266	+0.169	9.40	4.00	18.00 mg/l
Potassium	-0.226	+0.019	1.23	0.97	1.96 mg/l
Ammonia	-0.214	-0.117	0.24	0.00	0.98 mg/l
Magnesium	+0.197	+0.113	1.43	1.13	2.56 mg/l
Calcium	+0.117	+0.227	2.06	0.92	7.73 mg/l
Total Hardness	+0.112	+0.134	11.80	7.56	25.94 mg/l
pH	+0.086	+0.031	7.01	6.3	8.7
Chlorophyll a	-0.082	-0.111	16.6	0.005	169.00 mg/l
Total Phosphate	+0.061	-0.078	0.19	0.02	1.03 mg/l
Iron	-0.031	-0.237	0.47	0.00	2.91 mg/l
Silicon	+0.007	-0.104	8.70	4.70	12.00 mg/l
Nitrate	-	+0.242	0.21	0.00	0.65 mg/l
Orthophosphate	-	+0.153	0.03	0.00	0.14 mg/l

seasonal pattern from a low of 1.18 mg/l in January to a high of 45.48 mg/l in August. Large variances in chlorophyll a concentrations were observed in the water column, and much of this variance was due in part to the large growths of *Spirogyra* growing on the bottom of the pond.

TABLE 13. SIGNIFICANT CORRELATIONS BETWEEN ENVIRONMENTAL PARAMETERS AND *POLYARTHRA VULGARIS* AT STATION 2

Environmental Parameter	Net Mesh (Microns) 35	75	Concentration mean	low	high
Photoperiod	+0.522*	+0.281**	12.14	9.67	14.73 hr
Temperature	+0.497**	+0.400*	11.99	0.20	26.00 C
Ammonia	-0.385†	-0.272†	0.38	0.00	1.27 mg/l
Sodium	-0.298†	NS††	4.60	1.72	6.12 mg/l
Total filtrable solids	-0.269†	NS††	48.06	1.00	216.00 mg/l

Significant levels:

*.005
**.01
†.05
††not significant

Rotifer Populations

A total of 29 species of zooplankton were identified during the study (Edmunds, 1974) and 21 of these were rotifers. *Keratella cochlearis* was the dominant rotifer species. *K. cochlearis, Kellicottia bostoniensis,* and *Polyarthra vulgaris* were the most common rotifer species and they were present all year.

Polyarthra vulgaris fluctuated seasonally (Figure 7). Populations were low from December through April with a major peak between May and July and a minor peak in the fall. In May it was the most prevalent rotifer. The highest density observed was 1115 *Polyarthra* per liter and this occurred at the 1.5 m depth in June. The lowest values were between 0 and 1 animal/liter. The population increased in May within 3 weeks after the temperature exceeded 15 C. When the temperature was below 10 C, populations were very low (Figure 7).

Relation between *Polyarthra vulgaris* and Environmental Parameters

In correlating chemical and physical data on water quality to *Polyarthra* collected with the 35 micron mesh net there were significant positive correlations with temperature, nitrate, and orthophosphate (Tables 11 and 13). Additionally there was a signficiant positive correlation with photoperiod. Significant negative correlations was obtained with dissolved oxygen, ammonia,

TABLE 14. NONSIGNIFICANT CORRELATIONS BETWEEN ENVIRONMENTAL PARAMETERS AND *POLYARTHRA VULGARIS* AT STATION 2

Environmental Parameter	Net Mesh (micron)		Concentration		
	35	75	mean	low	high
Silicon	-.215	-.138	9.05	5.00	19.00 mg/l
Iron	-.180	-.095	0.73	0.00	5.95 mg/l
Oxygen	-.168	-.168	7.64	0.00	13.00 mg/l
Carbon Dioxide	-.145	-.077	6.02	0.00	100.00 mg/l
Orthophosphate	+.096	+.093	0.04	0.00	0.24 mg/l
Sulfate	+.092	-.104	2.60	0.00	5.16 mg/l
Hardness	-.088	-.022	12.50	7.12	38.82 mg/l
Chlorophyll a	-.081	-.031	19.30	0.38	150.70 mg/l
Total Alkalinity	-.074	-.026	12.47	5.00	123.50 mg/l
pH	+.070	-.050	7.02	6.10	8.40
Total Phosphate	-.046	-.028	0.30	0.02	12.60 mg/l
Magnesium	-.036	+.037	1.41	1.07	3.07 mg/l
Potassium	-.034	-.025	1.25	0.92	3.06 mg/l
Calcium	-.029	+.027	2.02	0.92	6.99 mg/l
Nitrate	+.022	+.117	0.23	0.00	1.59 mg/l
Total Filtrable Soil Solids	-.161	-	48.06	1.00	216.00 mg/l
Sodium	-	-.141	4.60	1.72	6.12 mg/l

sodium, and total filtrable solids (Tables 11 and 13). Similar correlations were obtained for the 75 micron mesh net except the effects of nitrate, orthophosphate, sodium, and total filtrable solids were not significant. There were no significant correlations with pH, total phosphate, sulfates, calcium, magnesium iron, total hardness, potassium, silicon, carbon dioxide, total alkalinity, or chlorophyll a (Tables 12 and 14).

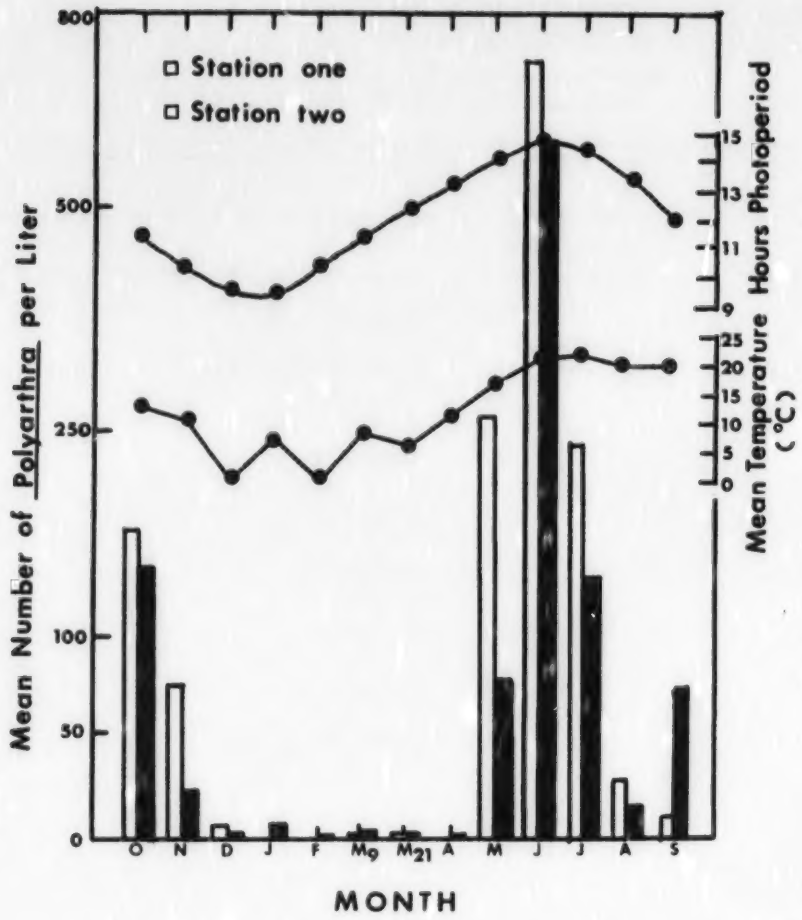

Figure 7. Seasonal Fluctuations of Photoperiod, Temperature and Mean Number of *Polyarthra vulgaris* Per Liter.

Using stepwise regression analysis the relationship between independent and dependent variables were identified (Tables 15 and 16). Data varied between stations and with net size. Five variables were identical from each station and net size. These were photoperiod, temperature, oxygen, nitrate, and total filtrable solids. In comparing the two tables, photoperiod accounts for 9.1 to 40.8% of the variation with better results obtained with the 35 micron mesh net. Variation due to temperature ranged from 11.0 to 21.1%. Oxygen varied from 0.3 to 13.0% and usually was under 4.0%. Nitrate accounted for 1.5 to 10.1% of the variation and total filtrable solids accounted for 2.6 to 20.4% of the variation.

Three relationships were identified only for the 35 micron mesh net. Orthophosphate accounted for 0.00 to 9.0% of the variation. Variation due to ammonia ranged from 4.6 to 14.8% and that for sodium ranged from 0.17 to 3.0%.

TABLE 15. STEPWISE REGRESSION ANALYSIS AND INCREASE IN COEFFICIENT OF DETERMINATION (R^2) FOR THE TOTAL NUMBER OF *POLYARTHRA VULGARIS* PER LITER WITH NO LAG
(Data are for the 35 micron net)

Station 1		Station 2	
Variable	R^2	Variable	R^2
Photoperiod	0.408	Photoperiod	0.224
Total Filterable Solids	0.204	Total Filterable Solids	0.179
Temperature	0.110	Temperature	0.164
Nitrate	0.101	Ammonia	0.148
Orthophosphate	0.090	Sodium	0.030
Ammonia	0.046	Nitrate	0.016
Oxygen	0.015	Oxygen	0.003
Sodium	0.002	Orthophosphate	0.00001
Total	0.726	Total	0.459

At the 0.05 level of significance the important parameters associated with 35 micron mesh net data were photoperiod and orthophosphate at Station One and photoperiod and temperature at Station Two. The eight variables identified in Table 15 account for only 45.9 to 72.6% of the variation.

Relationships between depth, alkalinity, magnesium, and silicon were associated with the 75 micron net data. Depth accounted for 4.7 to 5.4% of the variation while alkalinity ranged from 0.06 to 2.7%. Magnesium ranged from 0.14 to 1.1% of the variation. Silicon accounted for 1.1 to 1.9% of the variation. At the 0.05 level of significance temperature and silicon were most important at Station One, and temperature, magnesium, and nitrate were at Station Two. The nine variables identified in Table 16 accounted for 28.6 to 44.1% of the variation.

Potential invertebrate predators were identified and they include the rotifers *Synchaeta* sp. and *Asplanchna* sp., the copepod *Mesocyclops edax*, and the dipteran *Chaoborus* sp.

TABLE 16. STEPWISE REGRESSION ANALYSIS AND INCREASE IN COEFFICIENT OF DETERMINATION (R^2) FOR THE TOTAL NUMBER OF *POLYARTHRA VULGARIS* PER LITER WITH NO LAG
(Data are for the 75 micron net)

Station 1		Station 2	
Variable	R^2	Variable	R^2
Temperature	0.211	Temperature	0.158
Oxygen	0.130	Photoperiod	0.091
Photoperiod	0.130	Depth	0.047
Nitrate	0.057	Oxygen	0.037
Depth	0.054	Total Filterable Solids	0.026
Total Filterable Solids	0.028	Silica	0.020
Total Alkalinity	0.027	Nitrate	0.015
Magnesium	0.011	Magnesium	0.001
Silica	0.011	Total Alkalinity	0.001
Total	0.441	Total	0.286

DISCUSSION

Polyarthra vulgaris has been classified as a perennial, eurythermal species (Carlin, 1943; Pejler, 1957) which usually has a population maximum in late spring or early summer. The spring dominance of *P. vulgaris* in this study was similar to the observations of Carlin (1943), Pejler (1957), Beach (1960), and Abel (1974). Carlin (1943) and Edmondson (1965) concluded that the maximum occurs at a temperature range from below 15 to about 20 C and our data support these observations. Populations increased after the temperature reached 15 C and the maximum was observed in June when the temperature at the surface was 26 C. Carlin also suggested that there may be an autumnal maximum between 5 and 10 C. While there was an autumnal maximum observed in October this occurred when the mean temperature exceeded 10 C and as the temperature fell below 10 C so did the populations. Our laboratory studies also showed an inhibition of reproduction at 10 C (Figures 1 and 2) and Edmondson (1965) showed a depression of reproductive rate below 10 C. Temperature accounted for between 11.0 and 21.1% of the population variation. Positive relationships between temperature and the rotifers *Keratella cochlearis, Kellicottia longispina,*

and *Polyarthra vulgaris* (Edmondson, 1965) and the cladoceran *Daphnia ambigua* (Angino, Armitage and Saxena, 1973) have been found. Temperature explained 14.4% of *Polyarthra* variation (Edmondson, 1965), a value very similar to ours.

Because the populations of *Polyarthra* fell while temperature was conducive for reproduction other factors which could affect population dynamics were examined. One possibility is the delayed hatching of eggs. Another possibility is photoperiod which accounted for 9.1 to 40.8% of the population fluctuation. The positive correlation of *Polyarthra* populations and photoperiod may explain in part the June population peak, but it does not explain the smaller peak in the fall. In our culturing studies survival and egg production were enhanced by a photoperiod greater than 12 hr. Long photoperiods were associated with populations of *Daphnia schødleri* (Parker, 1966) and other species of *Daphnia* Angino, Armitage and Saxena, 1973).

Oxygen correlated negatively with *Polyarthra* populations and oxygen accounted for 0.3 to 13% of the variation. Pejler (1957) suggests that *P. vulgaris* is more "sensitive to deficiency of oxygen (and/or the conditions connected with it) than are other commoner eurythermal species." Initially, these results tend to be contradictory. The negative correlation in part may be illusory since higher oxygen concentrations were found during the cooler months when *Polyarthra* were in low numbers. Angino, Armitage, and Saxena (1973) found a similar negative correlation with *Daphnia ambigua* while Hazelwood and Parker (1961, 1963) found a positive correlation with *D. schlødleri*. Oxygen probably affects the survival of the early developmental stages of cladocera (Terao and Tanaka, 1928) and hatching of the rotifer *Brachionus* (Lite and Whitney, 1925). Angino, Armitage and Saxena (1973) suggested that low oxygen did not affect signficiantly the survival of *D. ambigua* because it only accounted for 0.5% of the variability. Our data demonstrated a variance as great as 13%. *Polyarthra* was rarely found in zones where the oxygen content was less than 5 mg/l. Aeration was important in culturing success and the animals died in stagnant conditions where oxygen was depleted. Our data support Pejler's (1957) observations on the sensitivity of *P. vulgaris* to low oxygen.

All correlations between nitrate concentration and *Polyarthra* numbers were positive and nitrate accounted for 1.5 to 10.1% of the variation. Nitrate did not show up as a significant variable until after a three-week lag with *Daphnia ambigua* (Angino, Armitage and Saxena, 1973) and after a one-week lag with *Diaptomus pallidus* (Armitage, Saxena and Angino, 1973). Our results suggest that nitrate may have an effect on *Polyarthra* but again the effect may be illusory. *Polyarthra* peaks close to spring overturn when there was an increase in the nitrate content of the water.

Total filtrable solids (TFS) were negatively correlated with *Polyarthra* numbers and TFS explained 2.6 to 20.4% of the variation. Seasonally the mean values for TFS were highest in late March (143.2 mg/l) and June and July (67.5 and 62.5 mg/l respectively) corresponding to periods of higher rainfall. The values of TFS increased near the bottom where there were few *Polyarthra*. Again the effect may be more illusory than real. *Polyarthra* are positively phototactic to weak light (Viaud, 1943) and as turbidity increases they probably

would move up the water column away from heavier particles and higher TFS values. Lastly, it is possible that TFS may effect light quality and quantity and its effect on the biology of the animal (see below).

Total alkalinity, magnesium silicon, and depth contributed to the variability of population data obtained with the 75 micron mesh net only. None of these were significantly correlated and their relationships varied from positive to negative (Tables 11, 12, 13, 14, 15 and 16). Borecky (1956) concluded that bicarbonate affected cladocera through its effect on food levels. Since *Polyarthra* may not eat many algae, the low variability for alkalinity and the insignificant correlations were not surprising.

Magnesium contributed 3.4% of the variation in populations of *Daphnia ambigua* (Angino, Armitage and Saxena, 1974) and 0.1 and 1.1% for *Polyarthra* (Table 16, this study). Magnesium is an essential enzyme activation (Prosser, 1973), and concentrations in Pandapas Pond probably were low enough to be favorable for survival and nontoxic as has been demonstrated by others for cladocera (Taub and Dollar, 1964; Crosby and Tucker, 1966). Only one slightly negative correlation with magnesium was found.

Silicon correlations were usually negative and they attributed between 1.1 to 1.95% of the variation. Silicon is related closely to diatom production and it is possible that dense diatom populations may inhibit *Polyarthra* populations.

Light affects the vertical distribution of *Polyarthra* and they are commonly found in the epilimion near the surface of a body of water (Berzins, 1958; Pejler, 1957; this study), and they are positively phototactic to moderate light (Viaud, 1943). Several hypothesis have been proposed for this vertical distribution of *Polyarthra vulgaris*.

Both Pejler (1957) and our study note that the rotifer is sensitive to low oxygen and *Polyarthra* may be near the surface because of higher oxygen concentration.

Pejler (1957) noted that the vertical distribution of *Polyarthra* may also parallel the vertical distribution of *Cryptomonas* in nature (Rodhe, 1955) and Carlin's data (Figures 49 and 100 in 1943) show variation. Edmondson (1965) obtained a significant positive correlation between *Cryptomonas* and *Polyarthra*. Based on our laboratory research this correlation may be illusory. *Cryptomonas* requires B_{12} and thiamin for growth (Hutchinson, 1967), and our culturing success of *Polyarthra* was impeded until B_{12}, thiamine, and other vitamins were added to the culture water. Perhaps both organisms were found together because of specific vitamin requirements.

Research on light effects on populations of *Daphnia pulex* (Buikema, 1972, 1973a, 1973b, and 1974) suggest that light itself may interact with biological processes and affect reproduction and growth. The vertical distribution of field rotifer population was always at moderate light intensities and laboratory populations aggregated at a light intensity of approximately 400 ft-c. It is possible that light intensity also has an effect on the biological processes of *P. vulgaris*.

Orthophosphate, ammonia, and sodium contributed to the variation of *Polyarthra* populations collected with the 35 micron mesh net (Table 15). Orthophosphate was positively correlated with *Polyarthra* populations and the effect was significant at Station One (Table 11). Again this correlation may be illusory because the rotifer peaks in spring when nutrients are released into the water. A negative correlation existed between phosphate and *Daphnia ambigua* (Angino, Armitage, and Saxena, 1973) and they suggest a possible toxic effect.

Ammonia accounts for 4.6 to 14.8% of the variation (Tables 15 and 16) and the correlation was significantly negative at Station Two. Ammonia was toxic to aquatic animals and this may alone explain its effect. An opposite effect was observed with *Daphnia ambigua* (Angino, Armitage, and Saxena, 1973).

The effect of sodium was negative and it accounted for 0.2 to 30% of the variability (Tables 11, 12, 13, 14, and 15). This relationship also may be illusory because sodium was highest in the winter when the rotifers were at a minimum. Sodium had no effect on populations of *Daphnia ambigua* (Angino, Armitage and Saxena, 1973) and its effect on the copepod *Diaptomus pallidus* occurred after a four-week lag (Armitage, Saxena, Angino, 1973).

Carbon dioxide, sulfates, potassium calcium, total hardness, pH, total phosphate, and iron were not significantly correlated nor did they appear in the stepwise regression analysis. Apparently *Polyarthra vulgaris* was insensitive to changes in these parameters over the range recorded in Pandapas Pond. The lack of a response to pH agrees with the conclusions of Pejler (1957) that pH was not an important determinant of *Polyarthra* distribution.

Pejler (1962) suggests that the appearance of *Polyarthra* may be explained by the abundance of algae in late spring or early summer. There was no significant correlation between chlorophyll a and numbers of *Polyarthra*. Our laboratory results were contrary to the observations of Edmondson (1965), Dieffenbach and Sachse (1911) and others since *Polyarthra* rarely ate algae. Interestingly, when the mean chlorophyll a values were the highest in July, August, and September the rotifer populations were declining. Also, phytoplankton biomass did not correlate with the population density of *Brachionus calyciflorus* (Halbach, 1972).

The possibility exists that there may be an antibiotic effect of algae on *Polyarthra*. In laboratory cultures if the bluegreen or green algae increased there was usually a concurrent decrease in *Polyarthra* population. If one compares Carlin's data for bluegreen algae and *Polyarthra vulgaris* an inhibition may exist (Figures 53 to 58, 100 and 101 in Carlin, 1943; Figures 145 and 146 in Hutchinson, 1967). An antibiotic effect between *Chlorella* and *Kellicottia longispina* has been suggested (Edmondson, 1965). *Branchionus plicatus* exhibits a decrease in survival in dense populations of *Chlorella* (Hirayama, Watanabe, and Kusano, 1973). Dense populations of *Chlorella pyrenoidosa* have a negative effect on *Branchionus calyciflorus* (Halbach, 1972; Halbach and Halbach-Keup, 1974).

Beach (1960) and Pejler (1961) note that declines in *Polyarthra* populations were concomitant with the appearance of a fungal parasite which penetrates the animals and eggs (Paterson, 1958, pers. observ.). Our observation suggests

that the sheath forming bacterium of the *Sphaerotilis-Leptothrix* complex also may have a negative effect on *Polyarthra*. While field animals were not observed to be parasitized, pond water returned to the laboratory would develop fungi and bacteria which hindered culture work. The potential impact when the female drops the egg prior to hatching is important. If spores contact the egg it will become infected. Efforts to identify the fungus beyond the chytrid group have not been successful (Martha Roane, pers. comm.).

Four potential invertebrate predators, *Synchaeta* sp., *Asplanchna* sp., *Mesocyclops edax*, and *Chaoborus* sp., were identified in the study. *Synchaeta* were present from February through May when *Polyarthra* populations were their lowest. None were collected when *Polyarthra* population peaks were dominant. *Asplanchna* sp. were only found in June at a density of six per liter at Station One and of two per liter at Station two. Average *Mesocyclops edax* densities were lowest (10-20/liter) during June through September when *Polyarthra* peaked. *Chaoborus* was seen only in August and September at densities less than 2/liter.

The breeding season for the bluegill *Lepomis macrochirus* was not known for Pandapas Pond but the impact of the bluegill larvae could be significant since they feed specifically on *Polyarthra* (Siefert, 1972). It is believed that predator impact was minimal on populations of *Polyarthra vulgaris*.

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APPENDIX

Preliminary procedures for batch culturing of *Polyarthra vulgaris*.

Because the rotifer has not been continuously cultured in the laboratory it will be necessary to periodically obtain animals from the field.

1. Collection - rotifers should be concentrated with a 35 micron mesh net. Cladocera and copepods should be removed as much as possible with a larger mesh net. Cultures should be started with an inoculum of about 1,000 rotifers per liter.

2. Handling - The rotifers should be handled with a 1 mm bore pipette or larger, and they should not be handled any more than necessary.

3. Containers - Glass containers with a minimum volume of one liter and a large surface to volume ratio should be used.

4. Culture medium - Use water from a natural source that contains *Polyarthra vulgaris*. The water should be filtered through a 10 or 35 micron mesh net to remove larger algae and animals.

   The culture medium should be partially replaced twice a week and totally replaced once a week.

5. Light - An incident illumination of 400 to 500 ft-c, a complete light spectrum and a 16L:8D photoperiod should be provided. G.E. cool white fluorescent bulbs are satisfactory.

6. Oxygen - Containers should be moderately aerated to maintain an oxygen concentration near 8 ppm.

7. Vitamins - Minimally the vitamins B_{12}, thiamine, biotin, and pantothenic acid should be supplied to the rotifer food organisms and added to the culture water. Commercial vitamin mixtures for pets, such as Vionate, can be used. To cultures containing five liters of medium, add one-half gram of Vionate after each partial change of culture medium and one gram after each complete change.

8. Food type - Feed a protozoan mixture of *Chilomonas paramecium, Cyathomonas truncatus, Bodo minimus, B. variabilis,* and *B. mutabilis*. These protozoans can be raised in a Purina Trout Chow medium that is fortified minimumally with vitamins B_{12}, thiamine, biotin, and pantothenic acid.

9. Food quantity - Feed 50 ml of this protozoan mixture to a 5-liter culture daily and the protozoan concentration should be around 300,000 protozoans per ml.

10. Temperature - Optimum temperatures were not specifically determined. Reproductive success was best at 20 - 22 C.

11. Antibiotic and parasitic agents - Observations should be made for fungi and bacteria on the rotifers and the presence of dense growths of green or bluegreen algae. Both were detrimental to the rotifer. These cultures should be restarted.

12. Population density - If the rotifer density decreases below 40 animales per liter the population may not recover. New cultures should be started.